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REMARKS

The Office Action of January 18, 2001 presents the examination of claims 28-34. Claims 28-31 and 33 are amended. Claim 35 is added for the Examiner's consideration. Support for claim 35 is found in the instant specification, specifically on page 8, lines 10 to 18. No new matter is inserted into the application.

It is submitted that the above-noted amendments raise no new issues and are proper under 37 C.F.R. § 1.116(a) because these amendments comply with the "requirements of form" discussed below in connection with the rejections under 35 U.S.C. § 112. Consequently, the amendments should be fully considered and entered by the Examiner. In any case, these amendments at least place this application into better form for consideration on appeal.

Specification

The Examiner requests that the trademarks used in the specification be capitalized and accompanied by generic terminology where possible. In response to the Examiner's request, Applicants amend the specification to capitalize the trademarks, as well as insert generic terminology for the trademarks.

Rejection under 35 U.S.C. § 112, first paragraph

Written Description

The Examiner rejects claims 28-34 under 35 U.S.C. § 112, first paragraph for allegedly containing subject matter not described in the specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner rejects to the term "gene" used in the claims and states that the rejection would be overcome by replacing "gene" with "DNA." In response to the Examiner's remarks, Applicants amend the claims accordingly. As the instant claims have been amended in compliance with the Examiner's suggestions, the instant rejection is overcome.

Enablement

The Examiner rejects claims 28-34 under 35 U.S.C. § 112, first paragraph for allegedly containing subject matter not enabled by the specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner asserts that the instant specification does not enable the use of any host cell deficient in growing ability in connection with the inventive method. Applicants respectfully disagree and submit that there is indeed sufficient guidance in the specification for one of skill in the art to make and use host cells without undue experimentation.

The methods recited in the instant claims utilize a host cell deficient in growing ability based on PPO activity. In other words, the host cell cannot oxidize PPO into protophyrin absent transformation with a vector comprising a DNA coding for PPO oxidase. The growth of the transformed host cell is indicative of PPO activity, when conducted under the culturing conditions recited in the specification. Specifically, when a host cell is transformed with the DNA fragment coding for PPO oxidase, the enzyme is expressed in the resulting transformant such that it confers to growth ability that would be otherwise deficient without introducing the DNA fragment into the host cell. The use of medium containing substantially no protoheme compounds also eliminates the possibility that there is not outside source of the heme compounds sufficient to provide the growth thereof.

In this regard, no undue experimentation is required to practice the methods recited in the claims. Specifically, no undue experimentation is required to find a host cell deficient in PPO activity (i.e. methods for determining PPO activity are well known in the art (see pages 12-13 of the instant specification)), transform that cell with a vector comprising a DNA encoding for PPO oxidase, and growing the cell on a medium free of protohemes. The specification teaches how to transform a host cell (page 10), as well as how to culture the host cell (see page 11). Applicants respectfully submit that for all of the

above reasons, the rejection for lack of enablement is improper and should be withdrawn.

Claim 33

Written Description

The Examiner rejects claim 33 under 35 U.S.C. § 112, first paragraph, for allegedly not being described in the specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner rejects claim 33 because the PPO genes from the various sources are not specifically disclosed. Applicants respectfully disagree. Again, Applicants emphasize that claim 33 is drawn to a method of using the PPO gene, rather than claiming the gene itself. Thus, Applicants submit that the written description provided in the specification is sufficient and, when conveyed to the skilled artisan, shows that Applicants are in possession of the claim method.

The methods recited in the claims comprise many steps that work together to evaluate the ability of a compound to inhibit PPO activity. The DNA fragment is described to the specificity that is needed for it to achieve the purpose of the claim. Even so, Applicants have amended the claims to recite a DNA fragment coding for the enzyme PPO which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability. Such a DNA fragment is involved in the steps in the

methods recited in the claims to achieve an evaluation of the ability of a compound to inhibit PPO activity. For all of the above reasons, Applicants respectfully submit that the instant rejection is improper and should be withdrawn.

Issues under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 29, 31, 33, and 34 under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claims 29 and 31

The Examiner apparently does not understand the terminator recited in claims 29 and 31. Applicants respectfully submit that the terminator serves to end transcription of the DNA fragment, which is explained on page 28 of the instant specification. A terminator and its function are readily known by one of skill in the art. Thus, the instant rejection is improper and should be withdrawn.

Claims 33 and 34

The Examiner rejects the recitation of "such as" in claim 33. In response to the Examiner's remarks, Applicants delete "such as" from the claim. Applicants also submit claim 35, directed to the deleted subject matter in proper form. Thus, the

instant rejection to claim 33, and dependent claim 34, is overcome.

Applicants respectfully submit that the pending claims satisfy the requirements of 35 U.S.C. § 112, second paragraph, and thus should be found allowable.

Conclusion

Applicants respectfully submit that the instant claims are fully in compliance with 35 U.S.C. § 112, first and second paragraphs. Further, all of the present claims define patentable subject matter such that this application should be placed into condition for allowance. Early and favorable action on the merits of the present application is thereby requested.

If there are any minor matters precluding allowance of the present application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at (703) 205-8000.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) month extension of time for filing a reply in connection with the present application, and the required fee of \$\$380.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional

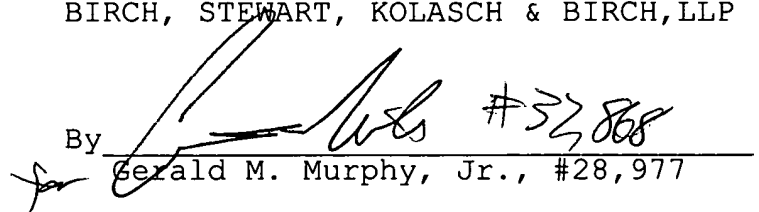
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fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17;
particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version with Markings to Show Changes Made



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Version To Show Marked Changes:

IN THE SPECIFICATION

Please replace the paragraph beginning on page 31, line 13, with the following rewritten paragraph:

--A library comprising λ ZAPII vector having inserted cDNA derived from a rat liver (manufactured by STRATAGENE) (hereinafter referred to as "rat cDNA library") was spread over several plates in NZCYM agar medium to amplify according to a method described in Molecular Cloning 2nd edition (authors: J. Sambrook, E.F. Frisch, T. Maniatis; Cold Spring Harbor Laboratory Press, 1989), 2.60-2.65 and phage particles were eluted with an SM buffer from the agar medium per each plate (hereinafter referred to as "amplified library"). A DNA was extracted from the amplified library using a DNA extracting kit (Lambda-TRAP PLUS: manufactured by Clontech) to prepare a phage cloned DNA. A polymerase chain reaction was performed using this phage cloned DNA as a template to amplify the DNA fragment. A reaction solution for polymerase chain reaction was prepared by taking 10 pmol of an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 3, 10 pmol of an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 4, 5 μ l 10 X PCR buffer (manufactured by TAKARA SHUZO CO., LTD), 0.25 μ l [TaKaRa Taq(] Tag DNA polymerase (TaKaRa Taq manufactured by TAKARA SHUZO CO., LTD), each 10 nmol of four kinds of nucleotides (dATP, dCTP,

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dGTP, dTTP: manufactured by TAKARA SHUZO CO., LTD), and 10ng of the phage clone DNA in a 0.5ml volume of a polymerase chain reaction tube and adding sterile distilled water to total 50 μ l. Each step of polymerase chain reaction was carried out under the following conditions: The first cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 2 minutes, and an extension step with a DNA polymerase holding a temperature at 72 °C for 3 minutes was performed once, and the second cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 1.5 minutes, and an extension step with a polymerase holding a temperature at 72 °C for 2 minutes was performed 34 times. After completion of the polymerase chain reaction, the reaction solution was analyzed on an agarose gel electrophoresis to select an amplified library from which about 700bp amplified DNA fragment is detected.

Please replace the paragraph beginning on page 33, line 18, with the following rewritten paragraph:

In order to clone a 5' upstream region of a PPO gene which is missing in the PPO DNA fragment obtained in Example 1, a polymerase chain reaction was performed using as a template a DNA extracted from a rat cDNA library to amplify the DNA fragment. A reaction solution for polymerase chain reaction was prepared by

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taking 10 pmol of an oligobase having the nucleotide sequence shown by SEQ ID: No. 4, 10pmol T3 primer (manufactured by TAKARA SHUZO CO., LTD) 0.5 μ l [TaKaRa LA Taq()] long amplifying Taq DNA polymerase (TaKaRa LA Taq manufactured by TAKARA SHUZO CO., LTD), 5 μ l 10 X LA PCR buffer (manufactured by TAKARA SHUZO CO., LTD), each 20 nmol of four kinds of nucleotides (dATP, dCTP, dGTP, dTTP: manufactured by Clontech), and 10ng of phage cloned DNA in 0.5ml volume of a polymerase chain reaction tube and adding sterile distilled water to total 50 μ l. Each step of polymerase chain reaction was carried out under the following conditions: The first cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 2 minutes, and an extension step with a DNA polymerase holding a temperature at 72 °C for 3 minutes was performed once, and the second cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 1.5 minutes, and an extension step with a polymerase holding a temperature at 72 °C for 2 minutes was performed 34 times. After completion of the polymerase chain reaction, the reaction solution was filtered by [MicroSpin S-400HR (] a spin column (MicroSpin S-400HR manufactured by Pharmacia Biotech) to purify the DNA fragments amplified by the polymerase chain reaction.

Please replace the paragraph beginning on page 37, line 4, with the following rewritten paragraph:

A [An] polymerase chain reaction was performed using as a template a full length gene cDNA encoding the rat-derived PPO obtained in Example 2 and as a primer an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 5 and that having the nucleotide sequence shown by SEQ ID: No. 6 to amplify the about 1.5kbp DNA fragment encoding PPO. A reaction solution in the polymerase chain reaction was prepared by taking 10pmol of an oligonucleotide having the nucleotide sequence shown by SEQ ID; No. 5, 10pmol of an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 6, 0.5 μ l of [TaKaRa LA Taq()] long-amplifying Tag DNA polymerase (TaKaRa LA Taq manufactured by TAKARA SHUZO CO., LTD), 5.0 μ l 10 X LA PCR buffer (manufactured by TAKARA SHUZO CO., LTD), each 20nmol of four kinds of nucleotides (dATP, dCTP, dGTP and dTTP; manufactured by Clontech), and 10ng of a plasmid containing full length cDNA of the rat PPO obtained in Example 2 in a 0.5ml volume of a polymerase chain reaction tube and adding sterile distilled water to total 50 μ l. Each step in the polymerase chain reaction was performed under the following conditions: The first cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for two minutes, and extension step with a DNA polymerase holding a temperature at 72 °C for three minutes was performed once, and

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the second cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 1.5 minutes, and an extension step holding a temperature at 72 °C for 2 minutes was performed 34 times.

Please replace the paragraph beginning on page 38, line 2, with the following rewritten paragraph:

After the polymerase chain reaction, the DNA fragment amplified by the polymerase chain reaction was purified by filtering the reaction solution with [MicroSpin S-400HR(] a spin column (MicroSpin S-400HR manufactured by Pharmacia Biotech). A terminus of this DNA fragment was cut with restriction enzymes SacII and SmaI. On the other hand, pBluescript II SK+ (manufactured by Stratagene) was cut with restriction enzymes SacII and SmaI (both manufactured by TAKARA SHUZO CO., LTD.) and the 5' end was dephosphorylated with calf intestine alkaline phosphatase (manufactured by TAKARA SHUZO CO., LTD).

Please replace the paragraph beginning on page 42, line 6, with the following rewritten paragraph:

A polymerase chain reaction was performed by preparing a reaction solution using Advantage cDNA PCR kit (Clontech) according to the attached manual and repeating once a cycle

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comprising 94 °C for 1 minute and 70 °C for 4 minutes, four times a cycle comprising 94 °C for 10 seconds, then 70 °C for 4 minutes, five times a cycle comprising 94 °C for 10 seconds, then 68 °C for 4 minutes, and 25 times a cycle comprising 94 °C for 10 seconds, then 65 °C for 5 minutes, and an aliquot of the reaction solution was subjected to agarose gel electrophoresis to confirm that about 2kbp amplified fragment is obtained. Further, the excess primers in the reaction solution were removed by performing the manipulation using [MicroSpin S400HR column()] a spin column (MicroSpin S400HR Pharmacia Biotech) according to the attached manual and the manipulation was performed using TA Cloning Kit (Invitrogen) according to the attached manual to clone the amplified fragment into pCR2.1 plasmid.

Please replace the paragraph beginning on page 43, line 16, with the following rewritten paragraph:

A polymerase chain reaction was performed using as a template a full length gene cDNA encoding PPO derived from *Chlamydomonas reinhardtii* obtained in Example 9 and as a primer an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 13 and that having the nucleotide sequence shown by SEQ ID: No 14 to amplify the about 2kbp DNA fragment encoding PPO. A reaction solution in the polymerase chain reaction was prepared by taking 10pmol of an oligonucleotide having the nucleotide sequence shown by SEQ ID: No. 13, 10pmol of an oligonucleotide

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having the nucleotide sequence shown by SEQ ID: No. 14, 0.5 µl of [TaKaRa LA Taq (] long-amplifying Taq DNA polymerase (TaKaRa LA Taq manufactured by TAKARA SHUZO CO., LTD), 5.0 µl of 10 X LA PCR buffer (manufactured by TAKARA SHUZO CO., LTD), each 20nmol of four kinds of nucleotides (dATP, dCTP, dGTP, dTTP: manufactured by Clontech), 10ng of a plasmid containing full length cDNA of *Chlamydomonas reinhardtii* PPO obtained in Example 9 in 0.5ml volume of a polymerase chain reaction tube and adding sterile distilled water to total 50 µl. Each step in the polymerase chain reaction was performed under the following conditions: After the first cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 2 minutes, and an extension step with a DNA polymerase holding a temperature 72 °C for 3 minutes was performed once, the second cycle comprising a denaturing step holding a temperature at 95 °C for 1 minute, an annealing step holding a temperature at 55 °C for 15 minutes, and an extension step holding a temperature at 72 °C for 2 minutes was performed 34 times.

Please replace the paragraph beginning on page 46, line 5, with the following rewritten paragraph:

A polymerase chain reaction is performed using as a template a full length cDNA of the rat-derived PPO obtained in Example 2, and using as a primer an oligonucleotide having the nucleotide

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sequence shown in SEQ ID: No. 7 and that having the nucleotide sequence shown in SEQ ID: No. 8 to amplify the about 1.5kbp DNA fragment encoding the rat PPO. The polymerase chain reaction is performed by adding 10pmol of an oligonucleotide having the nucleotide sequence shown in SEQ ID: No. 7, 10pmol of an oligonucleotide having the nucleotide sequence shown in SEQ ID: No. 8, 0.5 μ l of Advantage KlenTaq Polymerase Mix (manufactured by Clontech), 2.5 μ l of 10 X KlenTaq PCR reaction buffer (manufactured by Clontech), each 5nmol of four kinds of nucleotides (dATP, dCTP, dGTP, dTTP: manufactured by Clontech), and 10ng of the full length of cDNA of the rat-derived PPO obtained in Example 2 in a 0.2ml volume of a polymerase chain reaction tube to total amount of 25 μ l. Each step in the polymerase chain reaction is performed under the following conditions: After the first cycle comprising a denaturing step holding a temperature at 94 °C for 1 minute, and an annealing step and extension step with a DNA polymerase holding a temperature at 65 °C for 4 minutes is performed once, the second cycle comprising a denaturing step holding a temperature at 94 °C for 30 seconds, and an annealing step and extension step with a DNA polymerase holding a temperature at 65 °C for 4 minutes is performed 15 times. After completion of the polymerase chain reaction, the DNA fragment amplified in the polymerase chain reaction is purified by filtering the reaction solution with [MicroSpin S-400HR (] a spin column (MicroSpin S-400HR

manufactured by Pharmacia Biotech). After the end of this DNA fragment is made blunt with a DNA blunting kit (manufactured by TAKARA SHUZO CO., LTD), a phosphate group is added to the 5' end with T4 polynucleotide kinase (manufactured by TAKARA SHUZO CO., LTD).--

IN THE CLAIMS

Please amend the following claims:

28. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said [with a] vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell;

(2) culturing said transformant expressing said protoporphyrinogen oxidase [gene] DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.

29. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said [with a] vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell;

(2) culturing said transformant expressing said protoporphyrinogen oxidase [gene] DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit

the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

30. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said [with a] vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment which is a [gene] DNA capable of inducing the promoter of the first DNA fragment, and a promoter, wherein said promoter is not induced by the second DNA fragment but is functional in the host cell, are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase [gene] DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second

comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

31. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said [with a] vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment in which a [gene] DNA being capable of inducing the promoter of the first DNA fragment, a promoter, wherein said promoter is not induced by the DNA fragment but is functional in the host cell, and a terminator functionable in the host cell are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase [gene] DNA in a medium containing

substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

33. (Amended) The method according to any one of claims [claim] 28 to [or] 30, wherein [which is characterized in that] the protoporphyrinogen oxidase [gene] DNA is a protoporphyrinogen oxidase [gene] DNA derived from a group consisting of Dicotyledonous plants [such as *Arabidopsis*, soybean, oil seed rape, sugar beat, potato and tobacco], Monocotyledonous plants [such as corn, rice, wheat, barley, oat, rye, sugar cane and sorghum], algae [such as *Chlamydomonas reinhardtii* and *Chlorella*], mammals [such as mouse, rat, and human], fish [such as trout, bluegill, carp, cyprinodont, guppy, zebra fish and fathead minnow], and insects [such as fly, mosquito, cockroach, greasy grind, dragonfly and silkworm moth].

Please add the following claims:

--35. The method according to any one of claims 28 to 30, wherein the protoporphyrinogen oxidase DNA is a protoporphyrinogen oxidase DNA derived from a group consisting of *Arabidopsis*, soybean, oil seed rape, sugar beat, potato, tobacco, corn, rice, wheat, barley, oat, rye, sugar cane, sorghum, *Chlamydomonas reinhardtii*, *Chlorella*, mouse, rat, human, trout, bluegill, carp, cyprinodont, guppy, zebra fish, fathead minnow, fly, mosquito, cockroach, greasy grind, dragonfly and silkworm moth.--